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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :
MASATOSHI TOHATA, ET AL. : EXAMINER: POPA, I.
SERIAL NO: 10/578,613 :
FILED: MARCH 12, 2007 : GROUP ART UNIT: 1633
FOR: RECOMBINANT :
MICROORGANISM :

SUPPLEMENTAL REPLY BRIEF

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

In response to the *Advisory Action After the Filing of an Appeal Brief* dated August 31, 2011, the Appellants submit this Supplemental Reply Brief pursuant to 37 C.F.R. §41.43(a) and (b). The Advisory Action indicates by the checking of box 3 that the prior Reply Brief was entered. However, a supplemental response by the Examiner to the Reply Brief was attached to the Advisory Action. This supplemental response is a “supplemental examiner's answer responding to a reply brief” as described by 37 C.F.R. §41.43(a)(2) and the Appellants have the right to respond to this as provided for by 37 C.F.R. §41.43(b).

Lack of a Reasonable Expectation of Success

Ferrari Figs. 7 and 8

On page 2 of the Advisory Action the Examiner urges that Ferrari (WO ‘125), Figs. 7 and 8 do not evidence a lack of a reasonable expectation of success for the invention. The Examiner’s argument is that these figures show that deletion of genes *other than B. subtilis*

rocR, *slr* and *sigL* (the only genes under examination due to prior restriction¹) produce deletion mutants that produce more subtilisin (a heterologous protein) as determined by subtilisin activity. The Examiner reasons that if other kinds of deletion mutants increase heterologous protein expression, then deletion of *rocR* and *sigL* would have also been expected to increase heterologous protein expression, AA, p.2, lines 5-7:

Based on the data in Fig. 7 and 8 and Ferrari's disclosure, one of skill in the art would have reasonably expected that deleting *rocA*, *rocD* and/or *rocF* would also result in increased protein production.

However, this argument is not pertinent to the claims on appeal which are directed to microorganisms in which *rocR* and *sigL* are deleted. Figs. 7 and 8 are completely silent about the effects of deleting *rocR* and *sigL*. The same applies for the data in Fig. 8 which involves *sbo* and *slr* deletion mutants, not microorganisms deleted from *rocR* or *sigL*. These figures cannot provide support for the *prima facie* case and, in fact, teach away from the invention by showing that deleting different genes has different effects on heterologous protein expression. Moreover, Ferrari, refers to inactivating genes not necessary for strain viability (p. 2, lines 9-10), but is silent about whether *rocR* or *sigL* are such genes. Thus, Ferrari could never have provided a reasonable expectation of success for increasing heterologous protein expression by deleting or inactivating *rocR* or *sigL* as required by the invention.

Genetic Complexity

In the second paragraph on page 2 of the AA, the Examiner argues that there is "no genetic complexity" because "the prior art teaches that *rocR* and *sigL* only control no genes other than the *rocABC* and *rocDEF* genes" and

that based on the art as a whole, one of skill in the art would have known that knocking out *rocR* or *sigL* would only inhibit *rocABC* or *rocDEF*

¹ Contrary to the statement on p. 2, lines 8-9 of the AA, the claims on appeal are limited to microorganisms where *rocR*, *slr* or *sigL* are deleted or knocked-out, see OA dated January 7, 2010, lines 3-4 from bottom of page 2. However, since *slr* was deleted from the list of genes in claim 1, the claims on appeal read on microorganisms deleted or knocked out for *rocR* or *sigL*.

(i.e., arginine degradation) and achieve the desired and predictable result of accumulating arginine within the cell for enhanced protein synthesis.

The Examiner has made several incorrect presumptions above. First, the Examiner has not addressed the effects caused by deletion or inactivation of genes whose functions have not been completely elucidated. The data reported for genes functionally linked to *sigL* or *rocR* is partial, for example, Gardan, et al., Mol. Microbiol. 24:825, page 825, 2nd col., 1st full paragraph indicates that “the protein encoded by *rocB* is of **unknown function**” (emphasis added). Therefore, at the time of invention one of ordinary skill in the art would not have been able to predict the effects of knocking out expression of RocB by inactivating *sigL* or *rocR*. Moreover, one of ordinary skill in the art would not have been able to predict the cascade of intracellular effects caused by knocking out the other genes described by Gardan, et al. in the paragraph mentioned above.

Debarbouille, et al. 88:9092 (1991) further illustrates the complexity of the effects of knocking out genes like *sigL*. Its abstract states “*sigL* mutants cannot grow when arginine, ornithine, isoleucine, or valine is the primary nitrogen source”. That is, ornithine, isoleucine and valine as well as arginine are not available in *sigL* mutants, indicating that the effects of inactivating *sigL* are too genetically complex to be caused simply by the inactivation of the *rocABCDEF* operon alone.

Moreover, there is no support in the prior art for the notion that knocking out *sigL* or *rocR* would “only” inhibit RocABC or RocDEF. The Examiner bears the burden of showing this, but has not done so and one of ordinary skill in the art would have never accepted this. It was well-known that knocking out a gene could cause unexpected effects on other genes or gene products. Therefore that the results of knocking out *rocR* or *sigL* could hardly have been expected due to genetic complexity.

Ferrari shows that *B. subtilis* has a complex chromosomal genome that includes “prophage regions, antimicrobial (e.g., antibiotic) regions, regulator regions, multi-contiguous single gene regions and operon regions” (p. 2, lines 21-23) and exemplifies numerous different genes of the *B. subtilis* chromosome (p. 2, lines 27-29). Furthermore, Gardan, Mol. Microbiol. 24:825 shows the complexity of the arginine degradation pathway activated by RocR, see p. 825, both cols. In view of this complexity, one of ordinary skill in the art would have never expected that deleting *rocR* or *sigL* would “only” affect the RocABC or RocDEF operons. Moreover, the Appellants show in the present specification that knocking out *rocR* or *sigL* surprisingly increases heterologous protein expression.


Next, the Examiner presumes that knocking out *sigL* or *RocR* would cause a cell to accumulate arginine by inhibiting arginine degradation by inhibiting the rocABC and rocDEF operons involved in arginine metabolism. These operons encode RocC and RocE which Gardan, Mol. Microbiol. 24:825, page 825, col. 2, lines 10-11 suggests are amino acid permeases that permit uptake of arginine into a cell. Based on this teaching one would not have been motivated to knock out *rocR* or *sigL* with the expectation of increasing heterologous protein expression by increasing intracellular arginine levels. Knocking out arginine permease expression would have been expected to block a cell’s ability to accumulate arginine. Thus, based on these teachings away from the invention, one would have never expected that knocking out *rocR* or *sigL* would have increased heterologous protein expression. For all of the reasons set forth in the Appeal Brief and the Reply Briefs, this rejection cannot be sustained.

RELIEF REQUESTED

The Appellants respectfully request reversal of the grounds of rejection above and the allowance of this application.

Respectfully submitted,

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A handwritten signature in black ink, appearing to read "Stephen G. Baxter", is written over a horizontal line.

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